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AN INVESTIGATION OF EXTRACELLULAR ELECTRICAL CURRENTS AROUND CYANOBACTERIAL FILAMENTS

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ABSTRACT

We have searched for currents through gliding filaments of the giant cyanobacterium, *Oscillatoria princeps,* as well as through two species of *Anabaena* and found none. Current loops associated with gliding (and which would therefore have dimensions of the order of a filament length) should have been detected if they had surface densities of 0.03 to 0.1 $\mu A/cm^2$ or more; while current loops through *Anabaena* heterocysts should have been detected if they had surface densities of the order of 1 to 3 $\mu A/cm^2$ or more. The relationship of these negative findings to earlier reports of large voltages along *Phormidium* filaments is discussed.

INTRODUCTION

Using a vibrating probe to measure extracellular electrical fields, it has been shown that many growing eukaryotes drive steady electrical currents of the order of 1 to 100 $\mu A/cm^2$ through themselves (Jaffe, 1982). However, the only direct indicator of such currents through prokaryotes lies in several reports of extracellular voltages along gliding filaments of the cyanobacterium, *Phormidium uncinatum.* In one setup, Häder (1978) allowed a single *Phormidium* filament (in distilled water) to glide across a tight constriction formed by nearly fusing the end of a glass capillary. Unilateral illumination then induced voltages of up to 10 millivolts across the constriction. Later, Murvanidze and Glagolev (1982) placed a bundle of about 20 filaments, also in distilled water, along a fine groove formed by scratching a piece of Plexiglas. Illumination of one end of the bundle—in some experiments followed by a regime of turning uniform illumination on and then off—yielded voltages of up to 20 millivolts across the groove.

Such voltages could have been generated by the photoinduction of currents through the *Phormidium* filaments. If they were, then the fields so produced might well be measurable with a vibrating probe, without constriction of these currents by a capillary or a groove, since voltages a million times smaller, *i.e.,* of the order of 10 nanovolts or more, can be reliably measured with a vibrating probe system (Jaffe and Nuccitelli, 1974).

Currents through gliding filaments would be relevant not only to the study of the photophobic responses of cyanobacteria (Häder, 1978); but also in the possible mechanism of gliding (Jaffe, 1984). We have looked for such currents in a species of *Oscillatoria* that has exceptionally wide—actually 35 μm wide—filaments and exhibits rapid gliding motility. We also investigated two species of *Anabaena,* one motile and one not. In *Anabaena* we also looked for currents which might be associated with heterocysts, peculiar nitrogen-fixing cells which differentiate from vegetative cells at regular intervals along a filament (Fay *et al.,* 1968). In various

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eukaryotes, extracellular currents play a role in differentiation; moreover, heterocysts lack photosystem II (Tel-or and Stewart, 1977), and it seemed possible that they might show differences in photosynthetically driven proton flow through their plasma membranes from those found in vegetative cells—differences that in turn would generate detectible extracellular currents.

Materials and Methods

Anabaena flos-aquae strain 1304/13f from the Cambridge Collection of Algae and Protozoa (CCAP) and *Anabaena cylindrica* strain CCAP 1304/2a were cultured as described by Armstrong *et al.* (1983) at 20°C under an incident light (approximately 1000 lux). Current density measurements were made with filaments suspended in the same medium diluted 50% with distilled water to give a resistivity of 35 K Ωcm. *Oscillatoria princeps* strain ID-9-Op (from Dr. R. W. Castenholz, University of Oregon, Eugene) was grown in medium D (Castenholz, 1981). Measurements of current density were made with filaments suspended in this medium at full strength, in this same medium diluted ten times with distilled water, and also in 1 μM $CaCl_2$, a medium providing higher resistivity (450 KΩcm).

Current-generated fields around filaments were investigated using the vibrating probe with the filaments placed in 35-mm diameter Petri dishes on 1-mm deep layers of 1% (w/v) Difco agar jelly covered by 3 mm of the appropriate medium and a 2-mm layer of an inert parafin oil. The agar was prepared in the same medium as the overlay. To facilitate adhesion to the agar, filaments were first allowed to stick to the agar jelly without any fluid overlay.

The vibrating probe system was a modified version of that described by Jaffe and Nuccitelli (1974). Among the modifications were the following: (1) the vibrating electrodes were made by electrochemically depositing gold and then platinum black onto the tips of parylene-insulated stainless steel electrodes made primarily for brain recording (from Microprobe Inc., Clarksburg, Maryland). (2) The reference electrode was a non-vibrating platinized platinum wire immersed in the medium about a centimeter away from the vibrating one. (3) No meniscus setter was used. Where necessary, meniscal noise was avoided by covering the aqueous medium with oil. (4) The outputs of the vibrating and reference electrodes went to a differential preamplifier. The bath was kept near ground potential using a second platinized platinum wire and a virtual ground circuit. (5) For measurements of the field components perpendicular to a filament, the electrode was vibrated along its shaft instead of across it. This arrangement allowed a closer approach of the probe tip to the bacterial filaments than the usual lateral vibration, since the insulation did not intervene between the platinum black and the living cells. A close approach during such radial vibrations was also favored by the use of an electrode with a relatively small platinum black tip–one only 6 μm wide by 8 μm long.

Unless otherwise stated, measurements were made with a system time constant T of 5 s, *i.e.*, the system output after a step change in input rose to half of its final value in 5 s.

Figure 1 shows a probe near the end of an *Oscillatoria princeps* filament.

The filaments and probe were observed with a Zeiss inverted microscope. The most critical observations were made with a 40× objective. Most of the observations on *O. princeps* were made at a lamp voltage that gave an irradiance of 36 Wm^{-2} on the specimen. This is equivalent to a light intensity of about 9 klux (see conversion table of Van Liere and Walsby, 1982) and is similar to the light intensity used by Häder (1978). Some of the observations on *Oscillatoria* and on *Anabaena*

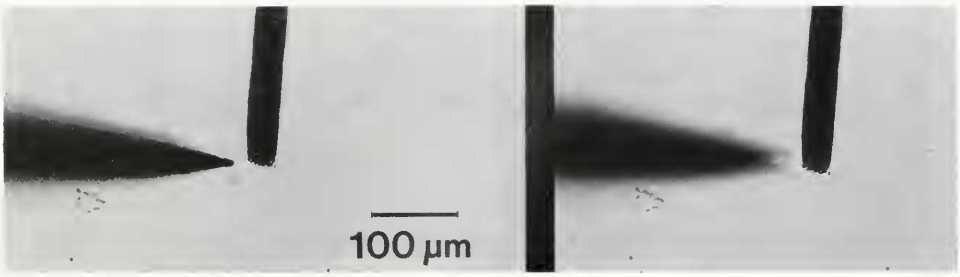

FIGURE 1. Photographs of a probe near the end of an *Oscillatoria princeps* filament. Left: probe static. Right: probe vibrating.

cylindrica and all measurements made with the photosensitive *A. flos-aquae* were made with a green filter in the lamp condensor which reduced the irradiance to 19% of the unfiltered value.

RESULTS

Measurements were made on two cyanobacterial species with typical widths: *Anabaena cylindrica* with filaments about 4 μm wide and *A. flos-aquae* with filaments 6 μm wide. They were also made on the exceptional filaments (36 μm wide) of *Oscillatoria princeps*. Since the measurements on the latter should be most reliable, they are described first.

Oscillatoria

A search for measurable currents was made on at least six different gliding filaments of *O. princeps*. The filaments generally glided at rates of about 1 to 5 μm/s during measurements with frequent reversals of direction. This search included measurements with the probe tip vibrating in the following directions and positions: (1) parallel to the filament and placed to one side of it with a minimal probe-to-filament gap of about 15 μm. (2) Parallel to and above the filament with a gap of about 40 μm. (3) Perpendicular and to the side with a 15-μm gap. (4) Oblique and above with a gap of about 50 μm. It likewise included measurements in various positions along the filament. In most cases we explored the vicinity of an entire filament (including its necridia) by letting it glide past a vibrating probe kept in one position except for slight adjustments to keep the probe-to-filament gap constant. The search also included measurement before, during, and after spontaneous reversals of the direction of gliding; measurements with the microscope light on or off and measurements during a shift from light on to off, or *vice versa;* as well as some measurements on filaments which were partially illuminated with the aid of the microscope light and the condensor diaphragm. The search likewise included measurements on filaments in medium D, in tenth strength D, as well as in a minimally conductive medium (1 μM $CaCl_2$ added to glass distilled water) which nevertheless supported continued gliding. These last measurements were done with a half time constant of 1.5 s instead of the usual 5 s.

In no case were currents detected. At the point of measurement we would estimate that the instrumental limits of detectability generally lay between 30 and 60 (or in a few cases 100) nA/cm^2, *i.e.*, 0.03 to 0.1 $\mu A/cm^2$. Tangential or parallel current densities associated with gliding would presumably fall off over dimensions

comparable to the lengths of the measured filaments. Since these filaments were the order of a millimeter or more in length, the tangential current densities should not have fallen off significantly from a filament's surface to the point of measurement; so tangential surface current densities of more than about 30 to 60 nA/cm^2 should therefore have been detected. Perpendicular density components of a 'gliding current,' on the other hand, would be expected to fall off inversely to the distance from a filament's mid line. Hence, perpendicular surface densities should have been 2 to 3 fold higher than at the measurement point, and ones more than about 100 nA/cm^2 should have been detected.

Figure 2 shows two sections of the original records, which illustrate these negative results with *Oscillatoria princeps*.

Anabaena

About ten filaments of *A. cylindrica* were explored in a similar way with similar negative results. However, they glided more slowly than *O. princeps* (0.2–0.3 μm/s instead of 1–5 μm/s). As a result, it was possible to vibrate the probe closer to the filaments. Probe-to-filament gaps as small as about 5 μm were often attained. Furthermore, *A. cylindrica* has heterocysts at about 100 μm intervals. Therefore, it was possible to search for special heterocyst currents in this species. These results

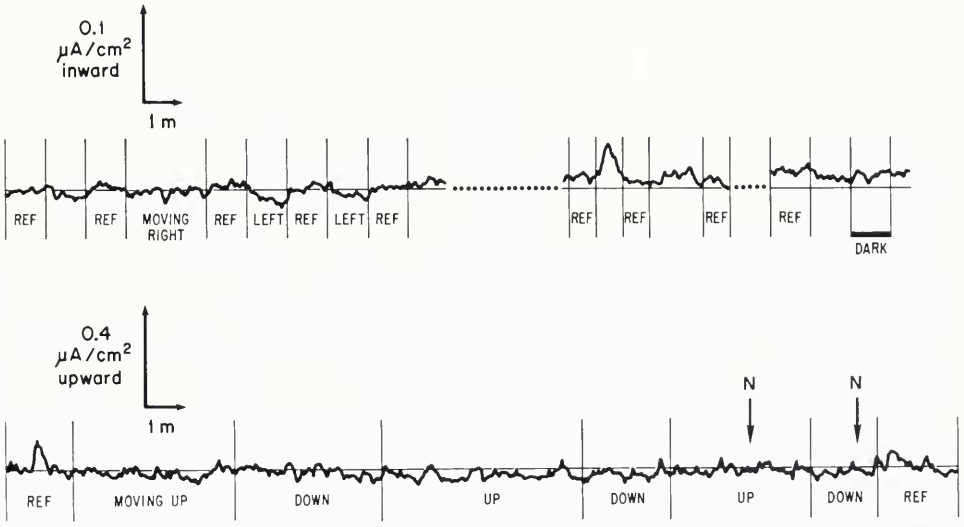

FIGURE 2. Illustrative records of the unsuccessful search for currents near gliding *Oscillatoria* filaments, in full strength medium D.

Top: probe vibrating *perpendicular* to filament 2 on 16 September 1984. The probe was to the side of the filament, about 5–10 μm away in its closest test positions (and 200 μm away in the reference positions marked 'REF'). The filament was observed to be gliding rightward or leftward during periods marked right or left; it was also moving at most other times, although the direction was not noted. The microscope light was on except where the chart is marked 'DARK.' During deleted parts of the record, the probe was not vibrating or had touched the filament.

Bottom: probe vibrating *parallel* to filament 3 on 17 September 1984. The probe was about 35 μm directly above the filament in its closest test positions and at this same height but 300 μm to the right in its reference positions. The filament was gliding horizontally 'upwards,' *i.e.*, away from the observer, or 'downwards' at about 4 μm/s in the periods marked up or down, respectively. N marks a point at which a necridium passed the probe.

were negative too. That is to say, currents of about 100 nA/cm^2 or more would have been detected in the region of measurement. Considered as a source or sink of current, each heterocyst is a roughly equi-dimensional object with a 3–4 μm radius. Currents emanating from a heterocyst should fall off roughly as the square of the distance from the cell's center. Considering the probe tip size of 6 $\times$ 8 μm, the minimum gap of 5 μm, etc., we estimated that perpendicular surface current densities as small as one to a few μA/cm^2 would have given detectable signals in the region of measurement. Since none were detected, we conclude that if there are special heterocyst currents, then their surface densities are less than 1 to 3 μA/cm^2.

Finally, we report some measurement made on the non-motile, 5–6 μm wide filaments of *A. flos-aquae*. At first we regularly observed apparent outward currents of a few hundred nA/cm^2 near (*i.e.*, about 5 μm away from) the filaments of this organism, but no corresponding inward currents could be found. Then we observed that these apparent currents were only generated by regions of a filament which were so loose that they visibly vibrated when the probe approached. No such signals were generated by well-stuck regions of a filament which did not visibly vibrate when the probe approached. Evidently, these apparent small outward currents are artifacts somehow produced by vibrating the filament. Perhaps these curious artifacts originate in electrokinetic effects produced by mechanically shearing a double layer at the filaments' outer surfaces. In any case, they are a warning against searching for currents in objects so light and so loosely tethered that the vibrating probe itself can vibrate them.

Discussion

The absence of detectable current-generated electrical fields around gliding filaments of *Oscillatoria* and of *Anabaena* raises the question of whether: (1) these filaments are fundamentally different from *Phormidium* filaments, (2) the voltages measured across *Phormidium* filaments were generated by extracellular currents which were (a) too transient or (b) too small to be detected by us, or (3) these voltages were not generated by electrical currents driven through the medium by the filaments, but in some other fundamentally different way.

The first possibility seems unlikely in view of the apparent similarity between different gliding cyanobacterial filaments. Moreover, a preliminary effort to measure current-generated fields near gliding *Phormidium* filaments using a vibrating probe system has also yielded negative results (Häder, pers. comm.).

Half times of the transient voltages recorded by Murvanidze and Glagolev were of the order of 10 to 30 s, so our system half time constant of 5 s should have allowed observations of comparable transients. Moreover, a crude calculation suggests that if the voltages which they recorded had been generated by currents through the filaments, then they would have been large enough to be detected by us. Suppose that their groove had a cross-sectional area of about 0.01 mm^2—as their reports suggest—and suppose that the medium in the groove had a resistivity of about 1 $\times$ 10^5 Ωcm. Then the resistance of their groove would have been about 1 megohm and the current per filament needed to generate 20 mV would have been about 1 nanoampere. This would have required a surface density of the order of 10 μA/cm^2 as it entered or left a filament section 0.5 mm long $\times$ 10 μm wide. There are many uncertainties in this calculation! Nevertheless, the figure is two orders of magnitude higher than our limits of detectability. This, in turn, suggests that the third possibility must be seriously considered.

How *could* the voltages recorded along *Phormidium* filaments have been

generated except by current flow through the filaments? One possibility is that they were diffusion potentials generated at liquid junctions outside of the filaments' plasma membranes. This seems particularly plausible when one considers that these voltages were recorded in "distilled water." In such a medium, the ionic strength and conductivity of the medium within the constrictions used—Häder's capillary or Murvanidze and Glagolev's groove—could well have been substantially raised by ions coming out of the filaments themselves. Under such circumstances, the liquid junction potentials within the constrictions may have been reduced relative to those at the ends of these constrictions, so that different liquid junctions at the ends would have generated substantial net voltages. In short, the extracellular *Phormidium* voltages recorded in the literature may indicate transient extracellular concentration gradients—as of pH, pCA, or sulfated polysaccharides in the slime—rather than electrical current flow through the filaments.

Altogether, our negative findings may be taken as evidence against an electrophoretic theory of gliding (Jaffe, 1984) and thus—by elimination—as being in favor of a sliding filament model (Castenholz, 1982).

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